RapidFinder™ Equine ID Kit

Part No: A15570

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1. Kit Description

Identification of meat species presence in food samples is an essential step in order to verify the origin and traceability of the used raw materials, as well as a necessary quality control for handling and cleaning processes of production lines.

RapidFinder™ Equine ID Kit allows determining the presence of DNA of horse (*Equus caballus*) in any food.

Equine DNA detection is done by real time PCR using two TaqMan®-MGB probes. One of them, labelled with FAM dye, specifically detects one mitochondrial DNA sequence of *Equus caballus*. The second probe is labelled with VIC and detects an Internal Positive Control, which is used to rule out inhibitors in the sample and check the correct functioning of the assay.

The detection limit has been calculated upon standard samples consisting of mixtures of raw horse meat and other species. **RapidFinder™ Equine ID Kit** can detect blends containing a percentage below 0.1% (w/w) of horse meat. The limit of detection in processed samples varies depending on the composition and food processing.

To ensure the representativeness of the results, we recommend the use of a DNA extraction method that allows you to process a large amount of sample (10-20 g). If you do not have a procedure with these features, we recommend the use of **GMO Extraction Kit** (Part No: 4466336).

2. Kit Contents and Storage

The Kit contents the necessary reagents to perform 48 reactions:

Reagents	Identification	Amount	Storage
Equine Master Mix	Red pad	378 µl	-20ºC
General Master Mix	White pad	630 µl	4ºC
Positive Control	Orange cap	60 µl	-20ºC

3. Equipment Requirements

In the following table the equipment requirements for using **RapidFinder™ Equine ID Kit** are shown:

	EQUIPMENT
1	Real-time PCR Thermal Cycler with channels for detection of FAM (520 nm) and VIC (550 nm)
2	Micropipettes (10 μl, 20 μl and 200 μl)
3	Table top centrifuge with adaptors for 96 well PCR plates and/or 0,2 ml tubes
4	Vortex

4. Consumables

Needed consumables are shown in the following table:

	MATERIALS
1	Optical 96-well reaction plates or 0,2 ml optical tubes
2	Optical adhesive film for 96 well plates or optical adhesive covers for 0,2 ml tubes
3	Disposable micropipette filter tips
4	1,5 ml sterile tubes
5	Powder-free latex gloves

5. Amplification Reactions Procedure

RapidFinder™ Equine ID Kit is designed to determine, in a single PCR reaction, the presence or absence of equine DNA and the internal positive control.

We recommend using, the positive control included in this kit for each run. This positive control contains 0,1% of equine DNA.

To estimate the amount of necessary reagents, we recommend make calculations taking into account the number of samples and controls to be simultaneously analysed, and then considering one more reaction, or increase a 10% the volume of each reagent.

The recommended protocol for preparation of amplification reactions is showed below:

1. Thaw the two Master Mix and the Positive Control vial
2. Vortex each reagent and keep cold.
3. In a 1.5 ml tube, add 7,5 µl of Equine Master Mix and 12,5 µl of General Master Mix for each reaction included in the same PCR run. Vortex and pipette 20 µl per well or tube of 0,2 ml.
4. Add 5 µl of each DNA sample at 10 ng/µl, into the appropiate wells. We recommend making each sample analysis in duplicate.
5. Add 5 µl of Positive Control and Negative Controls* into the appropiate wells.
6. Cover the well plate with optical film or the tubes with optical cover and spin in the centrifuge.

** We strongly recommend using an **extraction negative control** for each run of extractions carried out. This control consists in one tube to which no sample is added and which is summited to the same extraction process as the other samples. Likewise, we recommended using a **PCR negative control** for each PCR run; this tube contains no DNA but all PCR reagents.*

6. PCR Amplification Program

Amplification reactions must be summited to the following PCR program:

Temperature	Time	Cicles
95ºC	10 minutes	1
95ºC	15 seconds	36
60ºC	1 minute	

Note: This program has been validated on a StepOne Real-Time PCR System from Applied Biosystems. If you use another brand or model of thermal cycler, you may need the amplification program to be adjusted. Please contact our service department for advice.

7. Analysis of Results

Before analysing the samples results, you should check that the results obtained in the controls, is as expected:

- **Positive Control**: The result must always be positive in all amplification reactions, both in the FAM channel as VIC.
- **Negative controls**: Amplification must be only detected in the VIC channel. In this channel an internal positive control (IPC) is detected, which determines the absence of inhibition in the sample.

IPC

It must be checked that the IPC (VIC) is positive in all samples, with a Ct similar to the Positive Control. A negative result in the IPC indicates the presence of inhibitors in the sample. It should be noted that IPC result may be negative in samples where a lot of equine DNA (FAM) is detected, because the PCR reagents are exhausted before amplification of the IPC begins.

Equine

Amplification in the FAM channel indicates presence of equine DNA in the sample.

It is necessary to check if sample Ct is less than the $Ct_{cut\text{-}off}$ in order to determine if one reaction of amplification is positive. Any reaction of amplification with Ct upper than $Ct_{cut\text{-}off}$ may be considered as negative. The $Ct_{cut\text{-}off}$ is equal than the positive control Ct (0,1%) plus 3,32.

$$Ct_{cut\text{-}off} = 3{,}32 + Ct_{Positive\ Control}$$

Note: Any sample with a Ct equal than $Ct_{cut\text{-}off}$ contains approximately 0,01% of equine DNA.

In samples where no amplification in the FAM channel is seen, we can conclude that no equine DNA is detected or that its amount in the sample is below than the detection limit.

The following table shows graphically the results that may be obtained from one sample analysis, as well as the interpretation that should be done from the obtained result:

Master Mix Equine		INTERPRETATION
Equine	IPC	
-	+	No equine DNA is detected
+	+	Equine DNA is detected
-	-	PCR inhibitors presence in the sample*
+	-	Sample with big amount of equine DNA

** If presence of inhibitors in the sample is detected, we recommend checking whether there has been an excess of DNA in the reaction (the recommended maximum is 250 ng). If the amount of DNA is right, we recommend repeating DNA extraction. If the problem persists, please contact our technical department.*

The following table shows graphically the results that may be obtained from the analysis of different assay controls, as well as the interpretation that should be done from the obtained result:

Controls	Master Mix Equine		INTERPRETATION
	Equine	IPC	
Positive Control	+	+	Expected result
	-	-	PCR Amplification Failure[1]
Extraction Negative Control	-	+	Expected result
	+	+	Contamination in the equine DNA extraction procedure[2]
PCR Negative Control	-	+	Expected result
	+	+	PCR contamination with equine DNA[3]

Recomendations:

[1] **PCR Amplification Failure**: Check amplification program and configuration of fluorescence capture. Amplification failure may be due to a setup technical problem.

[2] **Contamination in the equine DNA extraction procedure:** Contamination may be due to some error made in the process of sample handling, reagents contamination, or environmental contamination. Check DNA extraction protocol, wipe the laboratory where DNA extraction process was performed and take care to avoid any contamination during sample homogenization. If necessary, use new aliquots of the reagents used in DNA extraction.

[3] **PCR contamination with equine DNA:** Contamination of PCR reactions may be due to an error made in the process of sample handling, contamination of the reagents or environmental contamination. Thoroughly clean the laboratory where the PCR process was performed, as well as equipment. If necessary, use new aliquots of the reagents used in the PCR. Prepare the PCR reaction containing the Positive Control last to avoid cross contamination.

8. Quantitative Analysis

In case of positive results, it is possible to determine the percentage of equine DNA versus total animal DNA present in one sample. We can use this kit in combination with **RapidFinder™ Quant Equine Set (Ref. A15579)**

Quantification assay is based in two absolute quantifications. One of these quantifications is performed making five serial dilutions from equine master mix (included in the RapidFinder™ Quant Equine Set) and doing PCR reactions using RapidFinder™ Equine ID Kit (mixing Equine Master Mix with General Master Mix). This quantitative assay determines the total amount of horse present in one sample. The second quantification is obtained doing PCR reactions using RapidFinder™ Quant Equine Set (mixing Animal Master Mix with General Master Mix). This quantification determines the total amount of animal present in one sample.

To design the animal TaqMan probe we selected a highly conserved mitochondrial genomic region.

For more information about quantitative analysis see the **RapidFinder™ Quant Equine Set** user manual (www.imegen.es), or contact our local distributor

9. Quality Control

All products commercialised by Instituto de Medicina Genómica has been subjected to rigorous quality control. **RapidFinder™ Equine ID Kit** has passed all internal validation tests, guaranteeing the reliability and reproducibility of each test.

You can refer to the Certificate of Analysis for your kit, entering the batch number of the product in the section Analysis Kits in www.imegen.es

10. Warranties and liabilities

Instituto de Medicina Genómica guarantees that all its products are free from defects, both in the used materials as in its manufacturing process. This warranty is extended to a period of one year from the date of shipment of the product, provided that storage conditions specified in this Manual have been observed. **Our products are designed for its use in testing of food and environmental samples.** The user of the product is responsible for validating the usefulness of the protocol proposed by Instituto de Medicina Genómica. These protocols are considered only guidelines. Instituto de Medicina Genómica does not offer any other warranty, either expressed or implied, which extend beyond the proper functioning of the components of this set. The only obligation of Instituto de Medicina Genómica, for the foregoing warranties, will be the replacement of the product or return the purchase price thereof, as desired by the customer, provided that proves the existence of a defect in materials, or in the development of the products. The Instituto de Medicina Genómica will not be responsible for any damages, direct or indirect, resulting in economic losses or damages resulting from the use of this product by the buyer or user.

11. Customer Support

For any inquiries about applications of this product or its protocols, please contact our Technical Department:

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